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The role of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in determining the prognosis of patients with testicular cancer (<https://www.balimedicaljournal.org/index.php/bmj/article/view/2977>)

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Bacteriuria in pregnancy in Sanglah Hospital: a descriptive study (<https://www.balimedicaljournal.org/index.php/bmj/article/view/3155>)

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Changes in plasma levels of IL-6 and D-dimer in high-risk thrombosis cancer patients undergoing chemotherapy (<https://www.balimedicaljournal.org/index.php/bmj/article/view/3162>)

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Predictive factors and the relationship between the early detection of osteoporosis and pathological fractures in Indonesian menopausal women (<https://www.balimedicaljournal.org/index.php/bmj/article/view/3258>)

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Comparison of serum and vitreous TGF- β 1 levels in proliferative diabetic retinopathy with and without panretinal photocoagulation laser therapy
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Habibah Setyawati Muhiddin, Rosmiaty Zainal Abidin, Budu ., Junaedi Sirajuddin, Itzar Chaidir Islam, Andi Muhammad Ichsan

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The effect of metformin on autophagy by LC3 expression in Type 2 Diabetes Mellitus (T2DM) human skeletal muscle cell culture
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Sigit Purbadi, Muhammad Yusuf, Wawaimuli Arozal, Aroem Naroeni, Hariyono Winarto, Andi Darma Putra, Gilbert Elia Sotarduga

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Change in ratio levels of KIM-1 / urine creatinine and increase serum creatinine levels in human immunodeficiency virus (HIV) patients receiving tenofovir-based antiretroviral (ARV) combination therapy
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I Dewa Gede Teguh Krisna Murti, I Ketus Agus Somia, I Gde Raka Widiana

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The outcome of hypospadias surgery and it's advanced treatment in Arifin Achmad General Hospital in January 2019 – December 2020
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Clinical and radiological profiles of metastatic brain tumor in Indonesia: A study at Dr. Soetomo Hospital, Surabaya
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The effect of workload and length of work on the occurrence of fatigue in workers in the informal industry
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Exploring the role of the combination of propolis and vitamin D3 on VCAM-1 and Caspase-3 expression in preventing atherosclerosis in chronic kidney disease rats
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Relationship between plasma adiponectin levels and cellulite
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The effect of Epigallocatechin-3-Gallate (EGCG) combined with low dose sorafenib in apoptosis and Platelet-Derived Growth Factor Receptor (PDGFR) expression in hepatocellular carcinoma rats
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The efficacy of probiotics supplementation on the lipid profiles of obese adolescents : a randomized trial
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Hematology profile of under five years old children suffered from acute diarrhea at Idaman Banjarbaru Hospital, Indonesia
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Role of malnutrition inflammation score and interleukin-6 on quality of life of regular hemodialysis patients
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Characteristics of COVID-19 patients with malignancies comorbidity in Sanglah General Hospital, Bali, Indonesia
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Early detection of elevated liver function test in drug-resistant tuberculosis with short term therapy and individual therapy
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The relationship of tumor necrosis factor alpha levels and neutrophils with skin wound age caused by sharp trauma
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Profile of melasma patients in dermatology and venerology outpatient clinic Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
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Correlation between antenatal magnesium sulfate (MgSO₄) total dose and delivery time interval with umbilical cord blood brain-derived neurotrophic factor (BDNF) levels as a neuroprotection strategy in preterm birth
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Muhammad Adrianes Bachnas, Sri Sulistyowati, Uchti Akbar

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The role of revascularization on short-term Heart Rate Variability (HRV) and Signal Averaged Electrocardiogram (SAECG) in Stable Coronary Artery Disease (CAD)
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Janry Pangemanan, Agnes Lucia Panda, Victor Giovannie Xaverison Rooroh, Evan Jim Gunawan

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Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism rather than homocysteine increase the risk of ischemic stroke-associated executive dysfunction
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Herpan Syafii Harahap, Muhammad Akbar, Andi Kurnia Bintang, Jumraini Tammasse, Andi Alfian Zainuddin

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Ima Kurniastuti, Ary Andini, Sabrina Ifahdini Soraya

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ORIGINAL ARTICLE

Self-acceptance of patients that received hemodialysis
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3106>)

Lono Wijayanti, Erika Martining Wardani, Difran Nobel Bistara, Siti Nur Hasina, Iis Noventi

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Ondansetron and metoclopramide: a comparative analysis of effectiveness and cost in hospitalized patients with hyperemesis gravidarum
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3223>)

Lonah, Purwastyastuti, Nafrialdi, Irwinda R, Via Dolorosa Halilintar

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User interface design of Be-Health application for children's learning with a gamification approach
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3111>)

Muhammad Wahyudi, Herwanda Ayu Destania, Rochmat Rizky Alfandi, Tri Sagirani

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Development of patient safety learning module based on problem based learning for nursing students at the College of Health Sciences
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The relationship between diabetes distress and HbA1C level in type 2 diabetes mellitus therapy patients: a systematic review
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Zefo Kiyosi Wibowo, Sony Wibisono Mudjanarko, Khairina Khairina

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Bioinformatics assessment on the potential of Lipoteichoic Acid (LTA) of Lactic Acid Bacteria (LAB) as topical therapy for inflammatory skin diseases
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The effect of antihypertensive monotherapy and combination on blood pressure in stroke patients
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Comparison of prognostic models for severe burn patients in an Indonesian tertiary hospital: retrospective study
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Eunice Geraldine Oenarta, Agus Roy Rusly Hariantana Hamid, I Gusti Putu Hendra Sanjaya, I Made Suka Adnyana, Tjokorda Gede Bagus Mahadewa, I Wayan Harimawan Agustinus

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Effect of proteasome inhibitor on serum 8-OHdG and aortic SOD2 in a rat model of atherosclerosis
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3126>)

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Patient preferences for surgery or non-surgery for the treatment of clavus and callus at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3264>)

Arisia Fadila, Iskandar Zulkarnain, Muhammad Yulianto Listiawan, Budi Utomo, Maylita Sari, Irmadita Citrashanty, Bagus Haryo Kusumoputro

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The relationship between catheter placement and the incidence of urinary tract infections in Condong Catur Hospital, Yogyakarta
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Soil worms (*Lumbricus rubellus*) as feed additives for piglets' growth, blood profile and immunomodulators
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Depot Medroxyprogesterone acetate reduces spermatogonia cells and spermatid cells in the seminiferous tubules of male mice
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Bagus Komang Satriyasa, I Gusti Ayu Widiyanti, I.B.G. Fajar Manuaba

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A structural model of Mapalus culture, health behavior and coronary artery disease incidence in the Minahasa ethnic community in North Sulawesi Province
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Jeini Ester Nelwan, Oksfriani Jufri Sumampouw, Adisti Aldegonda Rumayar, Franckie Maramis, Odi Roni Pinontoan, Ester Musa, Jansje Ticoalu, Edi Widjajanto

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A comparison of walking ability between the dynamic hip screw and cephalomedullary nailing fixations in intertrochanteric femur fracture
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The potential effect of intradermal Botulinum Toxin Type-A (BTA) injection to increase extended random skin flap survival
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Persistence of anti-Salmonella O9 IgM as measured by Tubex® TF may contribute to the over-diagnosis of typhoid fever in endemic areas
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3035>)

I Wayan Adi Pranata, Aly Diana, Marco R Heryanto, Nurhayati Lukman, Herman Kosasih, Hofiyya Djauhari, Deni PR Butarbutar, Susana Widjaja, Bachtli Alisjahbana

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Seroprevalence SARS-CoV-2 among the academic population of Universitas Gadjah Mada Yogyakarta
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Osman Sianipar, Umi Solekhah Intansari, Tri Ratnaningsih, Arum Tri Wahyuningsih, Fuad Anshori, Alfin Harjuno Dwiputro, Adika Zhulhi Arjana

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Early menarche, menstrual duration with dysmenorrhea in adolescents in Surabaya
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3109>)

Nety Mawarda Hatmanti, Yurike Septianingrum, Afita Riah, Firdaus, Ima Nadatien, Siti Maimunah

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The role of apparent diffusion coefficient in differentiating typical from atypical meningioma

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The effect of ACTH4-10Pro8-Gly9-Pro10 on neurotrophin-3 expression in Sprague Dawley rat on acute spinal cord injury
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3143>)

Made Gemma Daniswara Maliawan, Eko Agus Subagio, Budi Utomo, Muhammad Arifin Parenrengi, Asra Al Fauzi, I Ketut Sudiana

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High level of highly sensitivity c-reactive protein levels (hs-CRP) as a risk factor for preterm delivery
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Marthin Kolelupun, I Gede Putu Surya, I Nyoman Hariyasa Sanjaya, Tjok Gde Agung Suwardewa, I Wayan Megadhana, I Gede Mega Putra, I Nyoman Gede Budiana, I Wayan Artana Putra

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Retrospective Study on Very Early Relapse of Childhood Acute Lymphoblastic Leukemia at a Reference Centre in Indonesia
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C-Reactive Protein (CRP)/Albumin Ratio (CAR) pre-treatment as a predictive factor of radiological response after neoadjuvant chemotherapy in Locally Advanced Rectal Cancer (LARC) patients at Dr. Soetomo General Hospital, Surabaya, Indonesia
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Identification of PST 10 bacterial isolate with α -hemolysis characteristic isolated from pig's tonsil
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Characteristic of testicular torsion and predictors of testicular salvage: A retrospective study
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Alfryan Janardhana, Besut Daryanto, Budi Suwarno, Arif Rahman Nurdianto

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A novel scoring system to predict postoperative mortality after colorectal cancer surgery: a retrospective cohort study
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Correlation between Urea Creatinine Ratio (UCR) and lipid profile in COVID-19 patients
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The variability of temperature, rainfall, humidity and prevalence of dengue fever in Manado City
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/2722>)

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Devita Kurniawati, Budi Prasetyo, Hanindito Pandu, Arif Rahman Nurdianto

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Aqueous Humour Malondialdehyde Level as Oxidative Stress Marker In Types Of Glaucoma
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/2599>)

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CD44 expression as a potential favorable marker for prognosis in mucoepidermoid carcinoma of salivary gland
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/2793>)

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TNF- α serum Level between SARS-CoV-2 Infected Pregnant women with normal pregnant women in RSUD Dr. Soetomo Surabaya
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3377>)

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Combination effect of methotrexate with Narrowband Ultraviolet B (NB-UVB) phototherapy in psoriasis vulgaris patients in dermatology and venerology outpatient clinic Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3071>)

Emma Hidayati Sasmito, Iskandar Zulkarnain, Muhammad Yulianto Listiawan, Diah Mira Indramaya, Linda Astari, Budi Utomo, Afif Nurul Hidayati

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Application of Moringa Oleifera leaves extract cream inhibits paw edema in white male Wistar rat (*Rattus norvegicus*) induced by carrageenan 1%
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3384>)

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Human umbilical cords mesenchymal stem cells for kidney diseases
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3085>)

Dianita Halimah Harahap, Gampo Alam Irdam

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(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3343>)

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Medical education system in Coronavirus Disease 2019 (COVID-19) pandemic: what has been changed from the past era?
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3159>)

Ida Bagus Amertha Putra Manuaba, I Gede Putu Supadmanaba, I Gusti Ayu Sri Darmayani, Dwijo Anargha Sindhughosa, Made Violin Weda Yani

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The severe adverse event in a locally anesthetized circumcision: A case report of a breath-holding spell
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3379>)

Syifa Fauziah Fadhly, Irfan Wahyudi, Gerhard Reinaldi Situmorang, Arry Rodjani

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Diagnosing and treating Patent Foramen Ovale (PFO) from various manifestations in adults: case series
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/2779>)

Todung Donald Aposan Silalahi, Christopher Surya Suwita, Rosaria Oktafiani Darmawan, Benita Rosalie

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Gastrointestinal bleeding as a life-threatening complication of liver abnormality in a Turner syndrome patient
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3259>)

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Uncommon presentation of COVID-19 in ophthalmology: a case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3006>)

Cisca Kuswidyati, Klarissa Chrisalim, Andre

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Profuse haematochezia related to Crohn's disease: a rare case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3204>)

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CASE REPORT

Turner syndrome and tuberculosis in adolescent: a case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3243>)

Nyoman Titamarita Trisnawan, Florencia Christina Sindhu, Peter Prayogo Hsieh, Arya Putri Ratnasari, Alberto Afrian, Ancelia Limantara, Han's Christian, Made Ratna Dewi, I Wayan Bikin Suryawan

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Fatality in a pregnant woman with COVID-19 after a cesarean section: A case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3301>)

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CASE REPORT

Diagnostic challenges in Waldenström macroglobulinemia: a case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3160>)

Stella Pravita, Ugroseno Yudho Bintoro, Putu Niken Ayu Amrita

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Neonatal varicella: a rare case
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3060>)

Nanda Earlia, Wahyu Lestari, Fitri Dewi Ismida, Annisa Amalia, Aqil Yuliawan Tasrif, Mikyal Bulqiah, Dea Silvia Ramadana

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CASE REPORT

Diagnostic problems and management of pituitary gigantism leading to ischemic stroke and atrial myxoma in young adult patient: a case report
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3171>)

Nabilah, Sony Wibisono, Libriansyah, Joni Wahyuhadi, Muhammad Reza Arifianto

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Diagnostic problem on patient with tuberculous colitis mimicking Crohn's disease
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/3370>)

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The effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on neurotrophin-3 expression in Sprague Dawley rat on acute spinal cord injury



Made Gemma Daniswara Maliawan¹, Eko Agus Subagio^{1*}, Budi Utomo²,
Muhammad Arifin Parenrengi¹, Asra Al Fauzi¹, I Ketut Sudiana³

ABSTRACT

Background: Neurotrophic factors such as NT-3 play an important role in spinal cord regeneration, contributing to spinal cord regeneration assisting functional improvement in SCI. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, an analog of the N-terminal fragment (4-10) adrenocorticotrophic hormone, is one of the neuroprotective compounds that can increase NT-3 levels in brain ischemia. Therefore, we conducted a study with experimental animals to determine the effect of NT-3 expression on ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ administration in acute compression SCI.

Method: We used Sprague Dawley rats with acute compression SCI model using 20 gr and 35 gr aneurysm clips. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ was administered intranasally to the treatment group, and 0.9% NaCl was administered intranasally to the positive control group. Both of the groups then terminated at 3 and 6 hours.

Results: In rats with mild SCI that were given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10). Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ showed higher NT-3 expression than the group with 0.9% NaCl in mild and severe SCI.

Conclusion: Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ can increase NT-3 expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 in the group with ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ mild acute SCI was higher than in the group with severe acute SCI. Further research needs to be done to determine the neuroregeneration effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on SCI.

Keywords: spinal cord injury, central nervous system, neurotrophin-3.

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INTRODUCTION

Spinal cord injury (SCI) is an injury responsible for most disabilities, ranging from damage to sensory and motor functions to multiorgan dysfunction. The incidence of SCI in developing countries ranges from 13.1 to 163.4 per million population with prevalence rates between 490 to 560 per million population.¹ In Indonesia, there were 104 SCI cases registered at Fatmawati General Hospital in 2014, with the most common etiology being traffic accidents and falling from a height.²

The highest incidence of traumatic SCI occurs in men of productive age with an average age at the time of injury between 32 – 55.4 years, 37 – 47.9 years, and 26.8– 56.6 years, for North America, Europe,

and Asia, respectively.³ The high incidence of SCI at a young age certainly impacts the emergence of serious economic loss for families, communities, and countries. Functional defects caused by SCI significantly impact decreasing quality of life and patient life expectancy.⁴

Nerve cells in the central nervous system (CNS) in humans have a very limited regeneration response that does not allow for complete healing after injury.⁵ However, it is possible to develop neuroprotective therapies to maximize the functional integrity of the spinal cord remaining after injury.⁶ The most important thing in the treatment of spinal cord injury is how to achieve axonal regeneration in the damaged area.⁷ NT-3 is important for developing and maintaining

neuronal populations and promotes differentiation. Also, NT-3 is important in forming the substantia nigra and pyramidal pathways responsible for motor activity and have the best response in corticospinal axon regeneration compared to other neurotrophins, even considered superior.⁸

A neuroprotective compound, ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, also known as heptapeptide Semax an analog of the N-terminal fragment (4-10) of adrenocorticotrophic hormone, is known to enhance the transcription of NT-3 mRNA 24 hours after the ischemia of the rat brain cortex.⁹ ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ has also been shown to be effective in treating vascular disease, allergic and toxic inflammation, and partial atrophy

of the human optic nerve.¹⁰ Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ in rats with SCI showed an anti-inflammatory response expected to prevent secondary injury to nerve.^{11,12} Our study was aimed to determine the effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ administration on the expression of NT-3 in the spinal cord after mild and severe compression SCI.

METHOD

This study used a spinal cord injury model of male Sprague Dawley rats, weighing 250-300 g. Forty-five samples were divided into nine groups of five samples each. One group became the control group with the spinal cord was left uninjured as a baseline. The rest became the treatment group in which laminectomy at the level of 2nd thoracic vertebra was performed, followed by spinal cord compression using an aneurysm clip with a clamping force of 20 g for mild SCI (4 groups) and 35 g for severe SCI (4 groups) in 1 minute. The laminectomy site was then closed with sutures.

Each treatment group was divided into two subgroups. Positive control groups were given 0.9% NaCl intranasally, while the treatment groups were given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ intranasally at a 300 mg/kg dose. Then each group was divided again into two groups to be terminated, and then myelum transection was performed at 3 hours and 6 hours after compression. The preparations were fixed in formalin and examined for IHC. The NT-3 was calculated per 100 cells using associated anti-monoclonal antibodies and viewed with a light microscope using a 1000x magnification. Cells that showed positive expression gave the results of a brown color image in the cell cytoplasm (Figure 1).

Data collection is carried out in a controlled environment, assuming that all conditions are managed equally and controlled. Data of NT-3 level was presented in a relative expression graph. Data normality analysis was performed using Shapiro-Wilk, and then the collected data were analyzed using the nonparametric Kruskal-Wallis test with Mann-Whitney difference test.

RESULTS

The result of the normality test with the Shapiro-Wilk method showed abnormal data distribution with the Kruskal-Wallis test found significant differences ($p < 0,001$) between groups (table 1). In rats with mild SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the median NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10).

Mann-Whitney test was carried out to pinpoint the differences between groups. Overall NT-3 expressions are shown in figure 2. Except between the control group and group administered with NaCl 0,9% 3 hours, the median expression of NT-3 was significantly different between all comparison groups. Overall, it appears that administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ induced higher NT-3 expression compared to NaCl 0,9%. Also, the expression of NT-3 appeared to increase over time in groups treated with NaCl 0,9% but significantly decreased overtime in groups treated with ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.

Higher expression of NT-3 was also achieved significantly in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups with severe SCI

compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$) as shown in table 3. However, the time factor appears to be less significant in severe SCI because no difference was found when comparing the group with NaCl 0,9% at 3

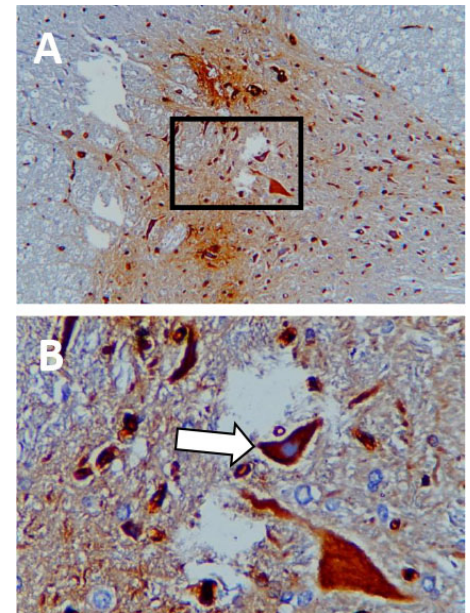


Figure 1. Neurons expressing NT-3 react with anti-NT-3, marked by brown cytoplasm (arrow) by IHC. A) 100x magnification. B) 400x magnification in the box marked area in figure A.

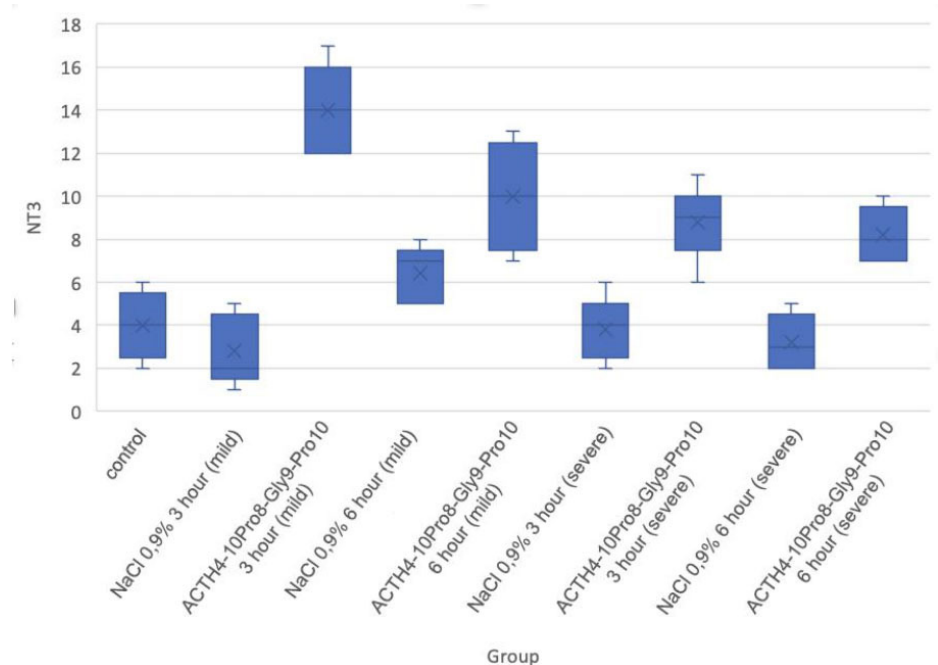


Figure 2. Median value of NT-3 expression.

hours and 6 hours and ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ at the same time frame.

Our study found significantly higher NT-3 expression in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with mild SCI at 3 hours compared to groups with severe SCI at both 3 hours and 6 hours shown in table

4. However, at 6 hours, the differences were not significant anymore. Therefore, it seems that the NT-3 inducing effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is optimum at 3 hours after administration in the mild SCI, and the effect is sustained or slightly reduced over time.

DISCUSSION

SCI causes an imbalance in the microenvironment that can exacerbate and accelerate nerve damage, impairing regeneration and functional recovery.¹³ The balance between proneurotrophins and neurotrophins is disrupted after SCI,

Table 1. NT-3 expression in all study groups.

Groups	NT3 Expression (cells/visual fields)			P
	Median	Min	Max	
Control	4	2	6	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 3 hours mild	14	12	17	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 6 hours mild	10	7	13	
NaCl 0,9% 3 hours mild	2	1	5	
NaCl 0,9% 6 hours mild	7	5	8	<0,001*
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 3 hours severe	9	6	11	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 6 hours severe	8	7	10	
NaCl 0,9% 3 hours severe	4	2	6	
NaCl 0,9% 6 hours severe	3	2	5	

Table 2. Comparison of NT-3 expression in rats with mild SCI.

Groups	Median	Groups	Median	p
Control	4	NaCl 0,9% 3 hours	2	0,242
		ACTH4-10 3 hours	14	0,009*
		NaCl 0,9% 6 hours	7	0,044*
		ACTH4-10 6 hours	10	0,009*
NaCl 0,9% 3 hours	2	ACTH4-10 3 hours	14	0,009*
		NaCl 0,9% 6 hours	7	0,014*
		ACTH4-10 6 hours	10	0,009*
ACTH4-10 3 hours	14	NaCl 0,9% 6 hours	7	0,008*
		ACTH4-10 6 hours	10	0,045*
NaCl 0,9% 6 hours	7	ACTH4-10 6 hours	10	0,033*

Table 3. Comparison of NT-3 expression in severe SCI.

Groups	Median	Groups	Median	p
Control	4	NaCl 0,9% 3 hours	4	0,831
		ACTH4-10 3 hours	9	0,011*
		NaCl 0,9% 6 hours	3	0,393
		ACTH4-10 6 hours	8	0,009*
NaCl 0,9% 3 hours	4	ACTH4-10 3 hours	9	0,011*
		NaCl 0,9% 6 hours	3	0,519
		ACTH4-10 6 hours	8	0,009*
ACTH4-10 3 hours	9	NaCl 0,9% 6 hours	3	0,008*
		ACTH4-10 6 hours	8	0,517
NaCl 0,9% 6 hours	3	ACTH4-10 6 hours	8	0,009*

Table 4. Comparison of NT-3 expression in mild and severe SCI.

Kelompok	Median	Kelompok	Median	p
ACTH4-10 3 hours mild	14	ACTH4-10 6 hours mild	10	0,045*
		ACTH4-10 3 hours severe	9	0,008*
		ACTH4-10 6 hours severe	8	0,009*
ACTH4-10 6 hours mild	10	ACTH4-10 3 hours severe	9	0,459
		ACTH4-10 6 hours severe	8	0,242
ACTH4-10 3 hours severe	9	ACTH4-10 6 hours severe	8	0,517

resulting in increased proneurotrophins and decreased neurotrophins, leading to cellular apoptosis, reducing synaptic plasticity, promoting the inflammatory response, and degeneration.¹⁴

NT-3, BDNF, and NGF expression decrease significantly as early as 6 hours after spinal cord contusion in rat model.¹⁵ Our study showed that NT-3 expression in rats with mild SCI was significantly higher in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$), as shown in table 2. Higher expression of NT-3 was also achieved significantly in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups with severe SCI compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$), as shown in table 3. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is known to act by binding to the melanocortin receptor, such as MC4R found in the spinal cord.¹⁶ In vitro studies on astrocytes showed that activation of the MC4R receptor can increase the expression of BDNF through the cAMP-PKA-CREB.¹⁷ A previous study in rats showed that administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ intranasally can increase BDNF expression in 3 hours after SCI.¹⁸ The mechanism for increasing NT-3 expression in this study has not been elucidated, but it is possible to have the same mechanism as the increase in BDNF.

Our study found significantly higher NT-3 expression in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with mild SCI than in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with severe SCI at 3 hours after injury ($p < 0,05$) as shown in table 4. Nerve damage that occurs after SCI can result from primary or secondary injury. More severe injury would result in more severe primary damage, causing a more severe imbalance at the tissue, cellular, and molecular levels,

which could affect the expression of NT-3. Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ in rats with SCI model also showed anti-inflammatory effects by reducing the expression of IL-1, TNF- α , NF-KB, neutrophil, and induced reduction in the apoptotic mechanism through increased ratio of Bcl-2/HSP70.^{11,12,19}

Our study's peak of NT-3 expression was shown in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group 3 hours after mild and severe SCI. Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is known to reach the rat brain within 2 minutes post-administration and has a concentration 10-15 times higher than intravenous administration²⁰. Another study in rats said that the nootropic and analgesic effects after intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ could be observed 15-30 minutes after administration.²¹ Human studies have shown that ACTH4-10 concentrations in spinal CSF increase within 10 minutes and peak within 30 minutes after intranasal administration, suggesting the possibility of using an extraneuronal pathway for the peptide to reach its target, pass through the intercellular gap in the olfactory epithelium and then diffuse into the subarachnoid space.²²

In this study, no locomotor assessment was carried out in rats, so the difference in the functional recovery effect of mild and severe SCI is not known after ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ treatment. However, one study showed improved BBB scores in rats with the SCI model after intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.² The receptor NT-3 also changes after the occurrence of SCI, so research on the TrkA, TrkB, TrkC, p75NTR, proneurotrophin, PC1, PC2, and furin receptors is also necessary to understand

more clearly the action of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.¹⁵

CONCLUSIONS

Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ increased NT-3 expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 was higher in the mild acute SCI group after administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ than in the severe acute SCI group. Further studies are needed to determine the neuroregeneration effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on SCI.

CONFLICT OF INTEREST

No competing interests were declared.

ETHICS CONSIDERATION

Ethics approval has been obtained from Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Airlangga University, Surabaya, with EC number 325-KE.

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AUTHOR CONTRIBUTIONS

All authors contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

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